in scope with these claims. The Examiner suggests that at the time of filing, the relevant art considered gene therapy as a whole to be unpredictable as modes of delivery that would provide efficient delivery and expression of genes encoding the therapeutic protein had not been developed. Further, the Examiner acknowledges that the specification is enabling for a method of performing ex vivo expansion of (a) a murine HSC comprising transducing the murine HSC with a nucleic acid encoding an ABC transporter and (b) a human HSC comprising transducing the human HSC with an ABC transporter and expanding the cell population for a period of up to 3 days, but suggests that it does not reasonably provide enablement for a method of ex vivo expansion of an HSC by transducing the HSC with an ABC transporter-encoding nucleic acid using any vector, and culturing the gene-modified HSC for more than 3 days to thereby expand the cell population.

The Examiner further suggests that there is no evidence supporting the assertion that human stem cells can be transduced with vectors at a low frequency, as evidenced by recent studies showing up to fifteen percent transduction in human patients. Further, the Examiner suggests that neither the prior art nor the specification demonstrate expansion beyond three days, and that in

an art which is unpredictable, the specification must provide specific guidance. Applicants respectfully disagree with the Examiner's characterization of the invention and respectfully request reconsideration.

The method of transduced HSC cell expansion described in the present application demonstrates that when transduction of stem cells is achieved, then amplification and expansion of the stem cells will occur for a period of time exceeding three days. Figure 2 demonstrates expansion of stem cells using the method of the present invention. The cells in this experiment were expanded for about 12 to 16 days in order to distinguish donor cells from recipient cells. The expanding cells with the control vector shown in 2A and 2B demonstrate that by day 50, the transplant cells are absent. In contrast 2C and 2D show an increased or expanded population of cells using the MDR vector. Figure 4 further demonstrates a quantitative analysis for repopulating cells. The degree of stem cell expansion is quantified as shown by the last six lanes on the right. In this experiment, two expanded populations of cells were mixed in equal amounts and implanted into mice. The MDR cells were the only cells to expand. (See attached Declaration at Paragraphs 3-5).

The success of the claimed invention does not depend on a high transduction frequency. Even if a low but detectable proportion of stem cells are transduced, it is the ABC transporter mediated expansion of these cells which allows for amplification and expansion, and provides a powerful tool for overcoming low gene transfer rates into stem cells. Further, although the transduction efficiency in mice is higher than the transduction efficiency in Rhesus monkeys and humans, the end result is the same. Once transduction of stem cells occurs, the transduced stem cells preferentially repopulate and expand, thereby increasing in number. Therefore, even a low transduction frequency will successfully increase the population of stem cells through expansion and repopulation. (See attached Declaration at Paragraph 12)

Contrary to the Examiner's suggestion that the success of gene therapy is unpredictable, the attached reference papers: Dunbar et al. 1996 Proc. Natl. Acad. Sci.,USA, Vol 93 pp 11871-11876; and Tisdale et al. 1998 Blood 92:1131-1141, show that at the time of filing the application, the use of gene therapy was appreciated by one of skill in the art to have progressed to the point of being successful in humans. Also, Applicants respectfully point out that

Figures 1-6 and pages 14-16 of the instant specification show expansion of stem cells for greater than a 3 day period of time.

The loss of unmodified stem cells in culture is clearly shared by mouse, Rhesus monkeys and humans. The observed measurable stem cell transduction responses of both the mouse and Rhesus monkey gene therapy studies are proof of predictable success. (See attached Declaration at Paragraph 6)

The expansion of transduced HSC cells described in the present application indicates that if transduction of stem cells can be achieved then amplification and expansion will occur for a period of time exceeding three days. (See particularly Figures 1-6 and attached Declaration at Paragraph 3-5). The present invention teaches a general method for repopulation and expansion of stem cells that can be applied to any species, including human. The examiner has failed to provide any reasonable basis to doubt that the stem cell expansion observed in cells and animal models in the instant invention would not also occur in humans.

Section 2107.02 of the MPEP states "if reasonably correlated to the particular therapeutic or pharmacological utility, data generated using *in vitro* assays or from testing in an animal model or a combination thereof almost invariably will be sufficient to

establish therapeutic or pharmacological utility for a compound, composition or process. Section 2107.02 of the MPEP further states "The applicant does not have to prove that a correlation exists between a particular activity and an assorted therapeutic use of a compound as a matter of statistical certainty, nor does he or she have to provide actual evidence of success in treating humans where such a utility is asserted. Instead, as the courts have repeatedly held, all that is required is a reasonable correlation between the activity and the asserted use *Nelson v. Bowler*, 626 F.2d 853, 857, 206 USPA 881, 884 (CCPA 1980).

In addition, the courts have held that it is only after the PTO provides evidence showing that one of ordinary skill in the art would reasonably doubt the asserted utility, does the burden shift to the applicant to provide rebuttal evidence sufficient to convince such a person of the invention's asserted utility. See *In re Bundy*, 209 USPQ 48, 51 (CCPA 1981).

Applicants respectfully assert that the PTO has not satisfied its initial burden, and Applicants should not have to substantiate a presumptively correct disclosure to avoid a rejection under the first paragraph of section 112, as the mouse studies alone should

be sufficient to satisfy Applicants' burden, as a matter of law. Applicants respectfully request reconsideration.

Claim 29 has been added to further clarify the invention as supported by the specification and by claim 2 as filed.

Withdrawal of this rejection is respectfully requested.

II. Rejection of Claims 1, 3-12 and 14 under 35 U.S.C. §102

The Examiner has maintained the rejection of claims 1, 3-12, and 14 under 35 U.S.C. §102(b) as being anticipated by McDonagh et al. (WO 93/24613). The Examiner has further rejected claims 1, 3-12, and 14 under 35 U.S.C. §102(e) as being anticipated by McDonagh et al. (US Patent No. 5,837,536).

The Examiner suggests that McDonagh et al. teach that HSCs expressing an ABC transporter were expanded 10 fold *in vitro*, and further how to make and use the claimed invention, as evidenced by Example 4. Applicants respectfully disagree.

The focus of the present invention is stem cell expansion and not the method used to introduce a gene into a cell.

Contrary to the Examiner's assertion, the expansion of cells reported in the McDonagh patent (US 5,837,536) at column 15, line 31 does not prove expansion of stem cells. CD34 cells are not

stem cells. Only a small proportion of the cells of a bone marrow sample (i.e. about 1 in 50,000) selected as CD34+ would actually be stem cells. (See attached Declaration at Paragraphs 7-8). Therefore this is not a pure stem cell culture at all. One of skill in the art can routinely obtain great increases of CD34+ cells in a culture, but the end result is that the stem cells suffer major losses. The loss of stem cells is in spite of major expansion of the CD34 cells. This concept is further exemplified in the attached reference, by Tisdale et al. entitled "Ex vivo expansion of Genetically Marked Rhesus Blood Progenitor Cells Results in Diminished Long-Term Repopulating Ability" (Blood (92) 4:1131-1141 (1998)). (See attached Declaration at Paragraph 9). Hematopoietic stem cells (HSC) from mammals including primates and particularly humans, were able to be successfully transduced using methods available in the art prior to May 28, 1998. Non-human primate HSCs, a very good model of human stem cells can be transduced with retroviral vectors at frequencies up to five percent. For example, see attached, Dunbar et al. 1996 Proc. Natl. Acad. Sci., USA, Vol 93 pp 11871-11876; and Tisdale et al. 1998 Blood 92:1131-1141 disclose successful transduction methods permitting gene transfer in HCSs with favorable transfer

efficiencies. (See attached Declaration at Paragraph 10). Further, it has also been shown that human stem cells can be transduced with vectors at a low frequency and recent studies have shown that transduction rates of up to fifteen percent can be achieved in human patients. (See attached Declaration at Paragraph 11).

Further, as noted in the previous amendment and response dated June 21, 2002, the promoter used by McDonagh et al. in this example does not and can not promote expansion in primitive stem cells. The attached papers by Stocking et al. entitled "Long Terminal Repeat Sequences Impart Hematopoietic Transformation Properties to the Myeloproliferative Sarcoma Virus" (Proc. Natl. Acad. Sci., USA 82:5746-5750(1985)); Tsuji et al. entitled "Retroviral Vector-mediated Gene Expression in Human CD34+CD38- Cells Expanded *in vitro*: Cis Elements of FMEV Are Superior to Those of Mo-muLV" (Hum. Gene Ther. 11:271-284(2000)), and Challita et al. entitled "Lack of Expression from a Retroviral Vector after Transduction of Murine Hematopoietic Stem Cells Is Associated with Methylation *in vivo*" (Proc. Natl. Acad. Sci, USA 91:2567-2571 (1994)) show that at the time of filing it was known that the Moloney promoter is not expressed in stem cells,

therefore, no expansion of stem cells would be expected to take place using this promoter.

Accordingly, the McDonagh et al. reference does not teach the present invention. Reconsideration and withdrawal of this rejection is respectfully requested.

III. Conclusion

Applicants believes that the foregoing comprises a full and complete response to the Office Action of record. Accordingly, favorable reconsideration and subsequent allowance of the pending claims is earnestly solicited.

Respectfully submitted,

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